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514413-3669**REMARKS**

Applicants request acceptance of the enclosed paper copy and computer readable form (floppy disk) of the Sequence Listing submitted concurrently under separate cover. The sequence disclosure fully complies with the requirements set forth in 37 C.F.R. § 1.821 to § 1.825. Pursuant to 37 C.F.R. §1.821 (g), the undersigned attorney hereby states that this submission does not contain new matter. Pursuant to 37 C.F.R. §1.821 (f), the undersigned attorney hereby states that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821 (c) and (e), respectively, are the same.

Claims 9, 11, 13 and 14 have been amended; claims 9 and 11-16 are pending in this application. No new matter is added.

Support for the amended claims can be found throughout the specification. Specifically, support for the amendment to claim 13 can be found in the paragraph bridging pages 9 and 10.

It is submitted that the claims, herewith and as originally presented, are patentably distinct over the prior art cited by the Examiner, and that these claims were in full compliance with the requirements of 35 U.S.C. §112. The amendments of and additions to the claims, as presented herein, are not made for purposes of patentability within the meaning of 35 U.S.C. §§§§ 101, 102, 103 or 112. Rather, these amendments and additions are made simply for clarification and to round out the scope of protection to which Applicants are entitled. Support is found throughout the specification and from the pending claims.

The Rejection Under 35 U.S.C. §112, 2nd Paragraph, Is Overcome

Claims 9 and 11-16 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Claim 9 has been amended to relate the "improved growth characteristics" back to the preamble, obviating the rejection with respect to claim 9 and dependent claims 11 and 12. Applicants believe that claims 13-16, which neither depend from claim 9 nor recite "improved growth characteristics", were inadvertently included in this rejection.

Applicants request that the various rejections under 35 U.S.C. §112, second paragraph, be reconsidered and withdrawn in light of these amendments and arguments.

The Rejections Under 35 U.S.C. §112, 1st Paragraph, Are Overcome

Claims 14-16 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking adequate written description. The Office Action claims that the claims were broader than the embodiment recited in the specification.

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The sequence of the leader peptide is given in the primary literature as cited (Wasmann et al., 1986), incorporated by reference into this application. In figure 5 of Wasmann et al., how a duplicate of the chloroplast leader sequence should look like and what is meant by duplication - namely:

MASMISSAVTTVSRASRGQSAAVASSAVTTVSRASRGQSAAVA (SEQ ID NO:

5)

in which the non-bold and underlined part represents the duplication of the non-bold and non-underlined part, and which clone is named pNi5125 (incorporated by reference into the pending application at page 15, lines 2-5). The subsequent paragraph in the application describes exactly the fusion of the so duplicated transit peptide sequence to the ASN-A gene.

The peptide sequence has been added to claim 14, and is adequately described in the specification.

Claims 9 and 11-16 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. As stated previously, the guidance present in the specification permits the cloning of further asparagine synthetase genes by the skilled artisan using standard plant molecular biology tools. If not available as a cloned nucleic sequence, such a gene can also easily be synthesized *in vitro* by applying standard means, as described in the preceding paragraph. For example, page 6, numbered points 1-3, briefly explains how the skilled artisan may proceed in order to identify further ASN-A genes from other organisms for making transgenic plants. The specification contains clear teachings on which classes of nucleic acid fragment to clone (tp and ASN) and methods for cloning them in order to obtain transgenic plants that exhibit the described features.

It should be noted that the claims now recite ammonium-specific asparagine synthetase, type A, specifically. Applicants should not be restricted to genes isolated from *E. coli* only, as similar genes can be isolated from other prokaryotic organisms, using techniques known to the skilled artisan, without undue experimentation.

Reconsideration and withdrawal of the rejections under the first paragraph of §112 are requested.

The Rejections Under 35 U.S.C. §103(a) Are Overcome

Claims 9, 11-13 and 16 were rejected under 35 U.S.C. §103(a), as allegedly being unpatentable over Coruzzi *et al.* (AG), in view of Dudits *et al.* (AH), Temple *et al.* (AP), and

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Della-Cioppa *et al.* Applicants assert that none of the cited references, either alone, or in any fair combination, serve to teach or suggest the presently claimed invention.

As it relates to Examiner's statement on page 5, 3rd paragraph of present Office Action, the demonstration of increased asparagine content (Example 8) and increased potato tuber weight (Example 7) in two plant species containing the construct as claimed is much more than an anecdotal statement, and it shows that there are clear effects on (a) the asparagine synthetase (AS) level in these transgenic plants and (b) the tuber weight of a storage organ of an important crop (potato).

Regarding the assertion on page 5 of the Office Action that "[t]here is no demonstration that plants also containing an antisense glutamine synthase (GS) gene showed any growth enhancement", Example 8 is directed to transgenic asparagine synthetase plants with reduced glutamine synthetase activity. Various parts of the application (see, for example, pages 4, 2nd and 3rd paragraphs, paragraph bridging pages 4 and 5, page 5, 2nd full paragraph, page 8, 1st paragraph and the paragraph bridging pages 9 and 10) describe reducing the glutamine synthetase level, i.e. with an anti-sense approach or by applying a chemical that inhibits the activity of glutamine synthetase.

Claim 13 now recites a construct containing a nucleic acid for anti-sense inhibition of glutamine synthetase. The making and use of such a construct and data on transgenic plants containing it are described in Example 4 and Example 6. Transgenic plants containing both types of DNA (1) the prokaryotic ammonium-specific asparagine synthetase (type A) coding region, and (2) an anti-sense RNA to an endogenous chloroplastic glutamine synthetase gene, both under control of a respective regulatory sequence, are described on page 17, 2nd paragraph of the description.

As was argued in the Amendment filed on May 23, 2002, Coruzzi refers to the suppression of more than one (if not all, in a non-selective manner) GS, which is, according to the disclosure, at least directed to cytosolic GS₁ and GS₃, as well as chloroplastic GS₂. This again clearly demonstrates that Coruzzi is not aware of the relevance of a selective knockout of the chloroplastic GS₂ as described in the present application. In no case, including in section 6.2.2 of Coruzzi, is the combination of a reduction of GS₂ and the timely adjusted up-regulation of ASN reported. The only related information is on page 48, lines 24-28: "Typically, plants co-suppressed for GS2 grow more slowly than wild-type and developed interveinal chlorosis (see

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Figure 10) due either to the toxicity associated with ammonia accumulation during photorespiration, or glutamine deficiency." There is no teaching, suggestion or motivation toward the combination of suppression of chloroplastic GS₂ and an increased level of AS in a single transgenic plant. The combination of suppressed GS₂ with prokaryotic ASN-A, which uses ammonium rather than glutamine for the production of asparagine (Cedar and Schwartz (1969) J. Biol. Chem. 244, 4112-4121) is also neither taught nor suggested. Contrary to this, Coruzzi reported on page 20, lines 22-28 the following:

~~"These plants having one altered enzyme also may be crossed with other altered plants engineered with alterations in the other nitrogen assimilation or utilization enzymes (e.g. cross a GS overexpressing plant to an AS overexpressing plant) to produce lines with even further enhanced physiological and/or agronomic properties compared to the parents."~~

Herein, Coruzzi *et al.* discuss simultaneous overexpression of GS and AS, but not about repression of GS and overexpression of AS.

Furthermore, Coruzzi *et al.* explain in more detail on page 21, lines 20-36:

"The present invention provides that engineering ectopic overexpression of one or more of those enzymes would produce plants with the desired physiological and agronomic properties. ... The engineering of enhanced expression of "root-specific" cytosolic GS (e.g., pea GS₁ is especially preferred." (Emphasis added. See also Table 2 on page 55 and Table 3 on pages 56/57 and related comments within the corresponding text.)

Coruzzi *et al.* actually teach away from the concept of the present invention. Consider the following statements: "these results show that the growth improvements are due to GS overexpression and not to the mere engineering of plants with the CaMV-35S GS constructs. For example, Z54-A1, which as been engineered with CaMV 35S-GS2 and was co-suppressed for GS expression, exhibited profoundly poor growth" (page 58, lines 4-12); and, "these results demonstrate that GS activity is a rate limiting step in plant growth as inhibition of this enzyme causes severe phenotypic effects on growth" (page 58, lines 9-12).

This is contrary to the present invention, where a reduction of GS activity is combined with the overexpression of a prokaryotic ASN in a transgenic plant, resulting in a significant increase in the observed tuber weight of the transgenic plants. Moreover, these transgenic plants show more vigorous growth and flower earlier than wild type plants.

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According to Coruzzi's assumptions, the inventive concept of an GS reduction and AS overexpression, as claimed herein, should not work at all.

Concerning AS overexpression, Coruzzi *et al.* refer to the ectopic expression of AS in a transgenic plant (ectopic as explained/defined in the Coruzzi specification to mean "in all cell types", see page 62, lines 16-17). In addition, Coruzzi *et al.* state that "the studies presented here examined whether the ectopic overexpression of AS in all cell types in a light-dependent fashion would increase asparagine production."

~~Contrary to this, the present specification clearly states and claims a prokaryotic AS~~ (ASN) which either is targeted to the chloroplasts or is expressed directly in the chloroplasts. This clearly means that the overexpressed ASN is present only in chloroplasts, and thereby only in green tissue, not in all cell types, as disclosed and intended by Coruzzi *et al.* Neither Coruzzi, Temple, Dudits, nor any combination of them teach or suggest that the combination of anti-sense GS₂ and overexpressed prokaryotic ASN would have a beneficial effect.


Coruzzi *et al.* further state: "It can therefore be anticipated that creating transgenic lines which express both GS and AS at high levels (by crossing AS and GS overexpressers) may have even more advantageous growth traits than either parent. In particular, the approaches disclosed here have the advantage that assimilation in transgenic lines will not be restricted to a few cell types, enabling available nitrogen in all plant cells to be utilized. The ectopic overexpression of both GS and AS in a single plant may have the advantage of avoiding glutamine accumulation..." (page 77, line 30 - page 78, line 8). Again, this is based on an increase in GS (which GS is not clear), rather than to a reduction in GS levels, either alone or in combination with overexpressed prokaryotic AS.

It is thus asserted that the claimed invention is not taught or suggested by the cited references, either individually or in any fair combination. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are respectfully requested.

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514413-3669**CONCLUSION**

Applicants believe that the application is in condition for allowance, and favorable reconsideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited. Alternatively, consideration and entry of this paper is requested, as it places this application into better condition for purposes of appeal. A Notice of Appeal, in triplicate, together with the required fee therefor, is filed concurrently herewith.

Respectfully submitted,

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514413-3669**VERSION WITH MARKINGS TO SHOW CHANGES MADE****In the Claims:**

9. (Four Times Amended) A process for the production of plants with improved growth characteristics, which comprises the following steps:
- b) transferring and integrating a nucleic acid encoding a polypeptide coding region comprising a prokaryotic ammonium-specific asparagine synthetase, type A, coding region linked to a chloroplast leader sequence for import of the asparagine synthetase into chloroplasts or plastids of a plant cell, wherein said nucleic acid is operatively linked to a regulatory sequence for expression in said plant cell;
 - b) transferring and integrating a nucleic acid for expression of an antisense chloroplastic glutamine synthetase RNA or portion thereof comprising transferring and integrating an anti-sense chloroplastic glutamine synthetase nucleic acid operatively linked to a regulatory sequence for expression of said anti-sense RNA or portion thereof in said cell to make a transformed cell; and
 - c) regenerating intact and fertile plants from the transformed cells, thereby producing plants with improved growth characteristics.
11. (Four Times Amended) A plant cell obtainable by the method of claim 9, comprising:
- a) a nucleic acid encoding a polypeptide coding region comprising a prokaryotic ammonium-specific asparagine synthetase, type A, coding region linked to a chloroplast leader sequence for import of the asparagine synthetase into chloroplasts or plastids of a plant cell, wherein said nucleic acid is operatively linked to a regulatory sequence for expression in said plant cell; and
 - b) a second nucleic acid for expression of an anti-sense RNA to an endogenous chloroplastic glutamine synthetase gene or portion thereof comprising a nucleic acid comprising an endogenous chloroplastic glutamine synthetase or portion thereof in an anti-sense orientation operatively linked to a regulatory sequence, said second nucleic acid providing reduced levels of endogenous chloroplastic glutamine synthetase activity upon expression of said anti-sense RNA in said cell.
13. (Thrice Amended) A gene construct comprising;

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- (c) a nucleic acid encoding a polypeptide [coding region] comprising a prokaryotic ammonium specific asparagine synthetase, type A, [coding region] linked at its N-terminus to a chloroplastic leader peptide sequence for import of the prokaryotic ammonium-specific asparagine synthetase, type A, into the chloroplasts or plastids of a plant cell, wherein said nucleic acid [and which construct] is operatively linked to a regulatory sequence for expression in said plant cell, and
- (d) a second nucleic acid for expression of an anti-sense sequence that encodes RNA molecule that is complementary to an endogenous chloroplastic glutamine synthetase gene or portion thereof, operably linked to a regulatory sequence for expression of the anti-sense RNA in the plant cell [wherein said plant cell exhibits the biochemical activity of the imported asparagine synthetase in its chloroplasts or plastids].

14. (Thrice Amended) A gene construct according to claim 13, wherein the prokaryotic ammonium-specific asparagine synthetase, type A, polypeptide coding region is linked at its N-terminus to a modified transit peptide having an amino acid sequence MASMISSAVTTVSRASRGOSA AVASSAVTTVSRASRGOSA A VA (SEQ ID NO: 5) [coding region from the small subunit of the Ribulosebiphosphate carboxylase from pea comprising a duplication of 20 amino acids from said transit peptide coding region].